09/237,291

Filing Date:

January 25, 1999 Response to May 2, 2008 Office Action

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1-17 (Canceled)

18. (Currently amended)A method for obtaining genetically modified modifying human

pluripotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous

gene with a population of human pluripotent hematopoietic stem cells cultured with fibronectin

and in the presence of an effective amount of a mpl ligand and a flt3 ligand, each ligand provided

in a concentration range of about 0.1 ng/mL to about 500 ng/mL, wherein said vector is selected

from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral

vectors, wherein said human pluripotent hematopoietic stem cells are CD34⁺Thy-1⁺Lin⁻ cells and

can differentiate into any hematopoietic cell type, and wherein said concentration range does not

cause differentiation of the human pluripotent hematopoietic stem cells; and

b) obtaining said modified human pluripotent hematopoietic stem cells.

19. (Previously presented) The method according to claim 18, further comprising culturing the

population of human pluripotent hematopoietic stem cells in the presence of a c-kit ligand in a

concentration of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said

vector.

20. (Previously presented) The method according to claim 19, further comprising culturing the

population of human pluripotent hematopoietic stem cells in the presence of a interleukin 3 (IL3)

in a concentration of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said

vector.

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21-22 (Canceled)

23. (Currently amended) A method for obtaining-genetically modified modifying human pluripotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human pluripotent hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of thrombopoietin (TPO), a flt3 ligand (FL), and interleukin-6 (IL-6), wherein the TPO, FL and IL-6 are each provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL and wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors, and wherein said human pluripotent hematopoietic stem cells are CD34⁺Thy-1⁺Lin⁻ cells and can differentiate into any hematopoietic cell type, and wherein said concentration range does not eause differentiation of the human pluripotent hematopoietic stem cells; and

- b) obtaining said modified human pluripotent hematopoietic stem cells.
- 24. (Previously presented) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of leukemia inhibitory factor (LIF) in a concentration range of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector.
- 25. (Previously presented) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of interleukin-3 (IL-3) in a concentration range of about 5 ng/mL to about 100 ng/mL prior to contacting said cells with said vector.
- 26. (Previously presented) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells is the presence of a c-kit ligand in a concentration range of about 5 ng/mL to about 100 ng/mL prior to contacting said cells with said vector.

27-30. (Canceled)

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31. (Previously presented) The method according to claim 23, wherein the effective amount of

TPO and FL individually is in the range of about 5 ng/mL to about 200 ng/mL and the effective

amount of IL-6 is in the range of about 10 ng/mL to about 100 ng/mL.

32. (Previously presented) The method according to claim 23, wherein the vector is a retroviral

vector.

33. (Previously presented) The method according to claim 23, wherein the heterologous gene is a

marker gene.

34. (Previously presented) The method according to claim 23, further comprising expanding the

modified human pluripotent hematopoietic stem cells.

35-36. (Canceled)

37. (Currently amended) A method of transducing human pluripotent CD34 Thy-1 Lin

hematopoietic stem cells, comprising:

a) obtaining a source of said stem cells, wherein said stem cells can differentiate into any

hematopoietic cell type;

b) culturing said cells with fibronectin and the cytokine thrombopoietin (TPO), flt3

ligand (FL), and interleukin-6 (IL-6), individually provided in the range of about 0.1 ng/mL to

about 500 ng/mL, wherein said concentration ranges do not cause differentiation of the human

pluripotent hematopoietic stem cells;

c) infecting the cultured cells with a retroviral vector including a polynucleotide sequence

encoding a heterologous gene; and

d) obtaining transduced cells wherein said gene is expressed.

38. (Previously presented) The method according to claim 37, wherein the TPO,FL and IL-6 are

individually provided in the range of about 5 ng/mL to about 200 ng/mL.

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39. (Previously presented) The method according to claim 37, further comprising culturing the

cells in the presence of leukemia inhibitory factor (LIF) in a concentration range of about 5

ng/mL to about 200 ng/mL.

40. (Currently amended) The method according to claim 37, further comprising culturing the

cells in the presence of IL-3 in a concentration of about 10 ng/mL to about 100 ng/mL, wherein

said concentration range does not cause differentiation of the human pluripotent hematopoietic

stem cells.

41. (Previously presented) The method according to claim 39, further comprising culturing the

cells in the presence of IL-3 in a concentration range of about 10 ng/mL to about 100 ng/mL.

42. (Previously presented) The method according to claim 37, wherein said IL-6 is in the range

of about 10ng/mL to about 100 ng/mL.

43. (Previously presented) The method according to claim 37, wherein the TPO is provided as a

mimetic.

44-45 (Canceled)

46. (Previously presented) The method according to claim 37, wherein the heterologous gene is a

marker gene.

47. (Previously presented) The method according to claim 37, wherein the heterologous gene is

a therapeutic gene.

48-51 (Canceled)

52. (Currently amended) A method for obtaining genetically modified modifying human

pluipotent hematopoietic stem cells, comprising:

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a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human pluripotent hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of a mpl ligand and a flt3 ligand, each ligand provided in a concentration range of about 0.1 ng/mL to about 500ng/mL, and optionally in the presence of one or more cytokines selected from: c-kit ligand in a concentration range of about 5 ng/mL to about 200ng/mL, interleukin 3 (IL-3) in a concentration range of about 5ng/mL to about 200ng/mL, leukemia inhibitory factor (LIF) in a concentration range of about 5 ng/mL to about 200 ng/mL, wherein said vector is selected from a group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors, and-wherein said stem cell can differentiate into any hematopoietic cell type, and wherein said concentration ranges do not cause differentiation of the human pluripotent hematopoietic stem cells; and

- b) obtaining said modified human pluipotent hematopoietic stem cells.
- 53. (Cancelled) A method of promoting the expansion of a population of human pluripotent hematopoietic stem cells comprising culturing *in vitro* a human pluripotent hematopoietic stem cell population in a medium comprising fibronectin, and an effective amount of a mpl ligand and a flt3 ligand, wherein each ligand is provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL, wherein said human pluripotent hematopoietic stem cells are CD34*Thy-1*Lin-cells and can differentiate into any hematopoietic cell type, and wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells.